REPORT OF RESEARCH INVESTIGATIONS
for the
ACADEMIC YEAR 1948-49
and for the
SUMMER OF 1948

I. Subject: RF48:207 Genetics of bacteria.

II. Personnel and Other Support:

J. Lederberg, Ass't Professor of Genetics, Project Leader

N. Zinder Research Assistant (now N.I.H.)

D. Gordon Project Assistant

This and related projects are also being supported by a three-year grant-in-aid from the Rockefeller Foundation ($7500), which is primarily for the purchase of major equipment, 1948-1951, and by annual grants from the N.I.H. (ca. $3500) which cover costs of personnel, supplies, and some equipment for the Salmonella work.

III. Scientific Progress, 1948-49:

A. Salmonella. The enclosed mimeographed report was prepared for the N.I.H., but summarizes the work nearly to date. Since the report was prepared, (April-May, 1949), somewhat more definite evidence for genetic recombination in Salmonella typhimurium has been obtained, but the results are still obscured by the problem of lysogenicity as discussed therein.

B. Gene enzyme relationships in E. coli. The genetic determination of β-galactosidase in Escherichia coli has been studied. A large number of lactose-negative mutants have been isolated following ultra-violet irradiation. These have then compared genetically and physiologically. At least seven, and possibly as many as twelve genes have been identified which are necessary for the formation of this enzyme. As a background for more detailed studies,

the enzyme has been extracted from the cells, and its properties examined in vitro with the aid of a chromogenic substrate for galactosidase: o-nitrophenyl- -galactoside. This compound was kindly provided by Professor K. P. Link and Mr. M. Seidman of the Department of Biochemistry.

The extracted galactosidase shows no remarkable features. It appears to behave as a single enzyme, with a linear time course of action, and a linear response to dilution. It is not inactivated by dialysis, nor does it require phosphate or other inorganic ions for activity. No evidence for a coenzyme was found. However, the enzyme is stimulated by sodium ion, in contrast to a strong inhibition by rubidium ion, and a relative inertness of potassium, except in competition with the other alkali metals. A number of substituted ammonium ions are also inhibitory (competitive with sodium). A kinetic study of alkali metal inhibition suggests that it is largely competitive with substrate but also shows some "uncompetitive" inhibition, comparable to the inhibition of Atmungsferment by azide. The results might be interpreted, however, as indicating that the metal ions are bound to the enzyme at a site adjacent to, but not congruent with, that which binds the substrate.

Kinetic studies, involving measurements of velocity as a function of substrate concentration, fit very well to the Michaelis-Menten theory of uni-unimolecular complexes of enzyme and substrate. It has also been possible to measure the affinities of various analogous galactosides for the enzyme by measuring the extent to which they impede the splitting of nitrophenol from its galactoside. Except that lactose has a relatively low affinity (high dissociation constant) compared to other galactosides, it is immaterial to

reproduce the constants here. The interactions of these galactosides provide an exceptionally clear example of competitive inhibition, with which formulation, the kinetics of the inhibition has fitted well.

Glucose and other reducing sugars (fructose, maltose, etc., but not sucrose or trehalose) are apparently non-specific inhibitors of galactosidase, as they are effective only at high concentrations (ca M/10) and show essentially non-competitive kinetics. The lack of competition of glucose is of some importance, because glucose is an exceedingly effective inhibitor of adaptation to lactose.

β-Galactosidase is a strictly adaptive enzyme, produced by E. coli only after exposure to lactose or other galactosides. Cells grown on glucose or other sugars contain very small quantities of the enzyme, less than 1% of the wild type, and the amounts that they do contain may yet be a residual from previous exposure to lactose, and to some extent also, a result of adaptation to the nitrophenyl galactoside used to estimate its activity. The most certain method of producing adapted cells is cultivation in a lactose-peptone medium. However, cells harvested and washed from a glucose medium may be adapted by suspending them for three hours in a lactose-buffer solution. Contrary to published statements, adaptation is not stimulated by the addition of inorganic nitrogen sources, but may be accelerated 2- or 3-fold by the addition of amino acids. Adaptation can be demonstrated in resting, non-growing suspensions, but is more regular and vigorous when accompanied by multiplication of the cells. For reasons not yet understood, some batches of cells fail to adapt when exposed to lactose for short periods, which has hindered further quantitative work pending the clarification of the necessary conditions.

The effects of the gene mutations can only be understood well in relation to the normal process of enzymatic adaptation, on which much work must be done. It seems to be apparent already that there is no direct relationship between a single gene and the specificity of the enzyme finally produced, as already indicated by the very complex genetic control involving more than seven already identified genes. However, a start has been made in our attempts to determine how each of the various mutations affects the production of the enzyme.

The mutations which absolutely and completely prevent adaptation can be analysed no further at present. This comprises the Lac_2, Lac_4 and Lac_7 loci as now understood. Two of the other loci have been examined to some extent. Lac_1- proves not to be absolutely lactose-negative, but produces, under optimal conditions with lactose as the growth substrate, about 5% of the normal galactosidase activity. With butyl galactoside, however, adaptation proceeds very much further, and results in about 30% of full activity. This difference in galactosidase activity is reflected in the rate at which lactose if fermented and results in this paradox: cells of the mutant grown on lactose ferment lactose much more slowly than they do if grown on butyl galactoside. The Lac_1- mutation, therefore, has not modified the "specificity-determination capacities" of the cell, but much more restrictedly, the capacity of the enzyme adaptation mechanism to respond to one substrate as compared with another.

Another mutation, Lac_3-, was found to affect not only lactose-fermentation, but also the utilization of glucose, and maltose. One such isolate proved to be distinctive in its temperature sensitivity: viz., it behaved like the normal at 30°, and like the mutant at 40°. Within this temperature interval, there are distinct thresholds for

the different effects, so that at 37°, for example, the culture would be classified as lactose-positive, glucose and maltose-negative. Preliminary experiments have shown that the effect of temperature is not on the enzyme, but on its formation, as shown by tests on cells grown and tested at different temperatures. Here again, we have evidence that the genetic effect is not on the integrity of a presumed "Template" but on some aspect of the complex adaptation mechanism. Competition between different substrates during adaptation shows that there is some element common to adaptation of different enzymes, which is supported here by the spreading or pleiotropic effect of one mutation on several enzymes.

Studies on maltose utilization by this mutant have resulted in a collaboration with Professor M. Doudoroff and his colleagues at the University of California. As mentioned the Lac_3^- mutant is unable to ferment either glucose or maltose. By selection on maltose medium, however, it is possible to derive strains which ferment maltose vigorously, but which are inactive on glucose. Doudoroff et al. showed that this anomaly is an expression of an amylomaltase mechanism, whereby n moles of maltose are polymerized into an amylose molecule and n glucose are split off. The amylose is then phosphorolysed to glucose-1-phosphate, thus shunting glucose itself which cannot be utilized. In dried preparations, this interpretation fits very well, and glucose is accumulated in requisite amount. It was observed here, however, that glucose is not accumulated during the fermentation of maltose by living cells, although small amounts of glucose added to the mixture were untouched. Serious questions of internal organization of enzymes, permeability, etc., are raised by these observations, for which there is so far no satisfactory explanation.

(It is hoped that some clarification may come out of Professor Doudoroff's visit to this laboratory this summer.)

C. "Extracellular transfer of heritable factors." It was hoped initially to study the phenomenon of type transformation which Boivin had reported in strain "C" of E. coli. A few preliminary experiments, however, soon indicated that our cultures, labelled "C", received from Boivin did not accord with his descriptions. Attempts to communicate with him have been unsuccessful, owing, we believe, to his hospitalization. This project has, therefore, been held in abeyance until suitable material can be obtained.

In the phenomenon of lysogenicity, however, there are certain aspects which may bear very strongly on what may be going on in such verified transformations as those of the pneumococci.

During several years' handling, strain K-12 of E. coli gave no sign of contamination with bacteriophage. Fortuitously, a mutant of K-12 was isolated which revealed that K-12, and almost all of the mutant substrains that have been obtained from it, are tenaciously infected with a bacteriophage. This phage has no apparent effects on the carrier strain, and can be detected only with the help of the indicator strain, the phage-sensitive mutant. The latent, lysogenic phage was termed "lambda".

When lambda-sensitive cells are exposed to lambda, about 3/4 of the cells are lysed, and release an augmented titre of the phage. The remaining 1/4 of the cells are not lysed, but instead become immune carriers of the phage, indistinguishable from the original lysogenic K-12. The phage is rather tenaciously bound to the lysogenic cells, as full grown cultures of the bacteria contain only a very small count

(ca. 100/ ml)of free phage particles. In addition, attempts to disinfect the lysogenic bacteria by artificial means (heavy ultraviolet radiations, rapid culture at high temperatures, viristatic chemicals, etc.) have so far been unsuccessful.

At first sight, the only sign by which sensitive and carrier bacteria can be distinguished is their sensitivity to lysis by lambda. Subsequently, another phage, p20, was isolated, from about a thousand plaques tested from sewage, which although it does not induce lysogenicity, is blockaded by pre-infection with lambda. That is, cells which are uniformly sensitive to p20 can be transformed to resistants by infecting them with lambda. It should be noted that, although considerable lysis by lambda can be detected in solid medium by plate counts in liquid medium there is no perceptible change in turbidity to signalize the lysis. On theother hand, resistance to p20 can also be realized by a gene mutation, which turns out to be the same as confers resistance to phage T6. From the point of view of the alteration of phage-resistance, lambda might be considered as a genetic transforming principle. Lysogenicity is probably far more common among bacteria than is generally realized, and should always be considered in mixed culture experiments. However, there is no evidence that it has any bearing on genetic recombination as studied in K-12.

D. Segregation from the diploid heterozygote in E. coli K-12. Previous investigations had involved the isolation of prototroph haploid recombinants, which indicated a single linkage system in this bacterium. More recently, diploid heterozygotes have been isolated which undergo frequent segregation and reduction to haploids, which are restricted to being prototrophic. Preliminary studies

(as published) have indicated that the segregation ratios for various genetic markers are far from 1:1, and may deviate as far as 15:1. This is probably due to the occurrence of a lethal deficiency in one or both of the chromosomes of the heterozygote. That lethality accounts for the deviation from normal segregations is supported by the single studies which Dr. M. R. Zelle of Cornell University has been doing with our collaboration. He has been able to follow single-cell pedigrees under the microscope, and has found numerous inviable cells, usually in association with a haploid segregant. However, the mechanism by which these lethal factors are produced has not been established, nor has any technique yet been devised which will circumvent this perturbation, and permit of the detailed study of the genetics of this bacterium in the absence of the disturbed segregations.

IV. Summary.

The work on bacterial genetics may be summarized under the following headings:

A. Salmonella (now with N.I.H. support). Attempts to demonstrate the occurrence of gene recombination in Salmonella are being carried out by Mr. Zinder. Although some encouraging, but equivocal results have been obtained with S. typhimurium, the prevalence of bacteriophage lysogenicity in this group has so far interfered with a clear-cut demonstration. So far, sexual reproduction among bacteria has been demonstrated only in one strain of Escherichia coli. It is important to determine the prevalence of this phenomenon.

B. Gene-enzyme relationships. A number of different genes have been found to influence the formation of lactase. New methods have been worked out for assaying this enzyme, and its kinetics and inhibition have been studied. It has become apparent that there is not a "one-to-one" relationship

between gene and enzyme in this instance, and that if there is any unitary action of the gene in this system, it must be on components of the adaptive enzyme mechanism. These components have different specificity, and some mutations may affect several enzymes. Biochemical study of a maltose-positive, glucose-negative has brought forth evidence for a "direct" mechanism of maltose fermentation. involving polymerization of the maltose to starch, with subsequent phosphorolysis.

C. Lysogenicity in E. coli K-12. It was accidentally discovered that strain K-12 which had been used for recombination studies, is also an immune carrier of a latent bacteriophage. The properties of this system, which is analogous to kappa in Paramecium, suggest an interpretation of the transformation of serological type in pneumococci based on infection with a cytoplasmic "virus".

D. Aberrant heterozygotes in K-12. The isolation of the diploid phase in E. coli permits of the direct analysis of genic segregations. These segregations are disturbed by a lethal factor which has not yet been analysed. The analysis of the disturbance will enable a judgment as to the extent to which the chromosomal mechanisms of this bacterium are the same as in higher forms.

V. (a) Publications, appeared in print during 1948-1949.

1. Lederberg, J., Problems in Microbial Genetics, Heredity 2: 145-198 1948 (Sept.)

2. " Detection of fermentative variants with tetrazolium, J. Bact. 56: 695 1948 (Nov.)

3. " Gene control of b-galactosidase in Escherichia coli, (abstract) Genetics 33: 617-618 1948 (Nov.)

4. Lederberg, J., Aberrant heterozygotes in Escherichia coli, Proc. Nat. Acad. Sci. 35: 178-184 1949 (Apr.)

*5. Lederberg, J. and Zinder, N., Concentration of biochemical mutants of bacteria with penicillin. J. Am. Chem. Soc. 70: 4267-68 1948 (Dec.)

*6. Doudoroff, M., Hassid, W. Z., Putnam, E. W., Potter, A. L., Lederberg, J., "Direct" utilization of maltose in Escherichia coli. J. Biol. Chem. 179: 921-934 1949 (June)

*Reprints not yet available, will be forwarded when received.

VI. (b) Manuscripts prepared or in press.

1. Lederberg, J., Bacterial Variation. Ann. Rev. Microbiol. 3: In press.

2. " Isolation and characterization of biochemical mutants. Methods of Medical Research 3: In press.

3. " Heredity, Variation and Adaptation. Chapter in Werkman Wilson, "Bacterial Physiology", in MS.

4. " The -galactosidase of Escherichia coli K-12. In MS, to be submitted to J. Biol. Chem.